PB90-129545

Respiration and Osmoregulation of the Estuarine Crab 'Rhithropanopeus harrisii' (Gould) Effects of the Herbicide, Alachlor

Duke Univ., Beaufort, NC

Prepared for:

Environmental Research Lab., Gulf Breeze, FL

1989

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completin

1. REPORT NO.	2.	3. R PB90-129545
EPA/600/J-89/169		

TLE AND SUBTITLE	5. REPORT DATE
RESPIRATION AND OSMOREGULATION OF THE ESTUARINE CRAB RHITHROPANOPEUS HARRISII (GOULD): EFFECTS OF THE HERBICIDE ALACHLOR	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) D.W. Diamond, L.K. Scott, R.B. Forward, Jr, and W. Kirby-smith	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Duke University Marine Laboratory Beaufort, NC 28516	10. PROGRAM ELEMENT NO. / 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS U.S. ENVIRONMENTAL PROTECTION AGENCY ENVIRONMENTAL RESEARCH LABORATORY OFFICE OF RESEARCH AND DEVELOPMENT GULF BREEZE, FLORIDA 32561	13. TYPE OF REPORT AND PERIOD COVERED / 14. SPONSORING AGENCY CODE EPA/600/04

15. SUPPLEMENTARY NOTES

Comp. Biochem. Physiol. 93A:313-318 1989

16. ABSTRACT

The effects of a sudden decrease in salinity and exposure to sublethal concentrations of the herbicide, Alachlor, on osmoregulation and respiration of the crab, Rhithropanopeus harrisii, were studied. 2. Crabs were hyperosmotic regulators at salinities below 24 ppt and became hypoosmotic at higher salinities. Upon a salinity decrease from 20 to 1 ppt, crabs adjusted their haemolymph osmolality to a stable hyperosmotic level in 8 hr. Alachlor concentrations to 50 ppm did not affect this adjustment. 3. A salinity decrease from 10 to 0 ppt elevated V_{O2} and the critical oxygen tension. This response was unaffected by Alachlor concentrations as high as 25 ppm.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL INFORMATION SERVICE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	7
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE A02

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA/600/J-89/169

Comp. Biochem. Physiol. Vol. 93A, No. 2, pp. 313–318, 1989
Printed in Great Britain

0300-9629/89 $3.00 + 0.00
© 1989 Pergamon Press plc

RESPIRATION AND OSMOREGULATION OF THE ESTUARINE CRAB, *RHITHROPANOPEUS HARRISII* (GOULD): EFFECTS OF THE HERBICIDE, ALACHLOR

DAVID W. DIAMOND, LAURA K. SCOTT, RICHARD B. FORWARD JR and W. KIRBY-SMITH
Duke University Marine Laboratory, Beaufort, NC 28516, USA. Telephone: (919) 728-2111

(*Received* 27 *July* 1988)

Abstract—1. The effects of a sudden decrease in salinity and exposure to sublethal concentrations of the herbicide, Alachlor, on osmoregulation and respiration of the crab, *Rhithropanopeus harrisii*, were studied.

2. Crabs were hyperosmotic regulators at salinities below 24 ppt and became hypoosmotic at higher salinities. Upon a salinity decrease from 20 to 1 ppt, crabs adjusted their haemolymph osmolality to a stable hyperosmotic level in 8 hr. Alachlor concentrations to 50 ppm did not affect this adjustment.

3. A salinity decrease from 10 to 0 ppt elevated $\dot{V}_{O_2}$ and the critical oxygen tension. This response was unaffected by Alachlor concentrations as high as 25 ppm.

INTRODUCTION

The crab, *Rhithropanopeus harrisii*, is perhaps the most abundant crab in the upper reaches of estuaries along the southeastern United States (Williams, 1984). Runoff from agricultural lands following rainfall can lead to a rapid decrease in salinity in estuarine areas and exposure to products used in agriculture. The present study was initiated because large numbers (unquantified) of dead *R. harrissii* were found following runoff from an agricultural area that was recently treated with the herbicide Alachlor. Although many environmental factors could contribute to crab death, exposure to Alachlor and a salinity decrease were chosen for study.

The herbicide, Alachlor (2-chloro-2′-6′diethyl-N-(methoxymethyl) acetanilide) is the active ingredient (45.1%) in Lasso® produced by the Monsanto Agricultural Products Company (Monsanto, 1984). It is used as a preemergent inhibitor of annual grasses, broadleaf weeds and yellow nutsedge with corn and soybean crops.

Alachlor is very soluble in water (242 ppm) and is widely used in amounts of 1.68–4.48 kg/ha by the farming industry (Weed Sci. Soc. Amer., 1979). Previous research has found that Alachlor can remain in the soils for up to 1 year (Skaggs *et al.*, 1980). After heavy rainfall Alachlor can easily be leached from soils into drainage systems, which transport it to estuaries Concentrations of Alachlor in drainage waters have been measured at 0.078 to 2.7 ppm (Wauchope, 1978; Skaggs *et al.*, 1980).

A recent study of Alachlor effects on larval development of an estuarine crab, *Rhithropanopeus harrisii*, found that there was a reduction in survival and a lengthening of developmental time with an increase in concentration (Takacs *et al.*, 1988). Lasso® was slightly more toxic than technical grade Alachlor, and the LC_{50} values ranged from 10 to 27 ppm depending upon the exposure time. Effects were evident after as little as 12 hr exposure. Since these LC_{50} values exceed the upper levels measured in nature and adults usually have higher LC_{50} levels than larvae, adult *R. harrisii* will probably be exposed to sublethal levels of Alachlor in nature. Osmoregulation and respiration represent two physiological processes that could be affected by sublethal stress, and if altered, could contribute to an animal's death.

Osmoregulation is well studied among crustaceans (recently reviewed by Gilles and Pequeux, 1983). Using inorganic ion concentration as a measure of the relationship between hemolymph and sea-water osmolality, Smith (1967) suggested that *R. harrisii* was a hypersomatic osmoregulator at salinities between 1 and 23 ppt but became hypoosmotic at higher salinities. The present study determined this relationship by directly measuring sea-water and haemolymph osmolality and considered the effects of Alachlor upon acclimation to a sudden reduction in salinity.

Respiration is also well studied in crustaceans (recently reviewed by Cameron and Mangum, 1983; Vernberg, 1983). In estuaries, the oxygen concentration fluctuates widely throughout the 24 hr day, being highest during mid to late afternoon as a result of photosynthesis and being least at late night and early morning, due to the combined respiration of aquatic plants and animals (e.g. Warner, 1977; Kenney *et al.*, 1988). When the external oxygen tension is high many crustaceans exhibit independent weight-specific oxygen consumption rates ($\dot{V}_{O_2}$) and when external oxygen tension is low, dependent $\dot{V}_{O_2}$ occurs. The inflection oxygen tension for the switch from independent to dependent respiration is the critical oxygen tension (P_c; Hill, 1976). Both $\dot{V}_{O_2}$ and the P_c were used as an assay for the effects of Alachlor and salinity decrease upon respiration of *R. harrisii*. Results indicate that neither osmoregulation nor respiration were affected by sublethal concentrations of Alachlor.

MATERIALS AND METHODS

Rhithropanopeus harrisii (Gould) were collected without regard for sex or molt stage from the Neuse River, North Carolina from September to November. They were kept

unfed in 10 ppt sea-water in glass finger bowls (19 cm diameter) at a temperature of about 23°C. The water in the bowls was changed every 2 days, and all experiments were completed within 3 weeks of collection.

Herbicide preparation

Lasso®, an emulsifiable concentrate (EC) of Alachlor, was obtained commercially and used in the experiments because this form is slightly more toxic than pure Alachlor to *R. harrisii* larvae (Takacs *et al.*, 1988) and it is the form to which crabs are exposed in nature. A stock solution was made by pipetting 10 ml of Lasso® EC Alachlor (480 g/l) into a 1 l glass bottle and allowed to evaporate. Certified A.C.S. acetone was then added to give a final volume of 480 ml stock solution, which produced a nominal concentration of 10 mg/ml (10,000 ppm). The stock solution was stored in dark at 5°C. Unless otherwise specified in the remaining paper, the designation Alachlor is derived from Lasso®.

Standard dilution techniques were used to produce acetone dilutions of 2500, 1250 and 500 ppm (mg/l). To each 7 cm diameter glass finger bowl, 1 ml of dilution was pipetted and allowed to evaporate to dryness in a hood. The remaining Alachlor film was then resuspended in 50 ml of filtered (5 μm) sea-water at the test salinity to give final Alachlor nominal concentrations of 10, 25 and 50 ppm. These concentrations were used because the LC_{50} values for *R. harrisii* larvae with identical techniques ranged from 10 to 27 ppm (Takacs *et al.*, 1988); these values are sublethal for the adults and are in the same order of magnitude as the highest levels reported in nature (Skaggs *et al.*, 1980). Test crabs remained alive upon exposure to test concentrations of Alachlor. An acetone control was not run because larval development was not affected by acetone (Takacs *et al.*, 1988).

Osmoregulation experiments

An initial experiment determined the relationship between haemolymph osmolality and external salinity. Groups of five crabs were acclimated for one week to separate salinities ranging from 1 to 30 ppt at 4–5 ppt intervals. The different salinities were made up by mixing dechlorinated tap water with sea-water and were measured with a refractometer (A.O.). Approximately 10 μl of haemolymph was withdrawn from the base of the crab's walking legs using a 1 ml syringe with a No. 27 needle. Haemolymph osmolality was measured immediately with a vapor pressure osmometer (Wescor, Inc., Model 5100B). The osmolalities of the acclimation waters were measured with the same osmometer.

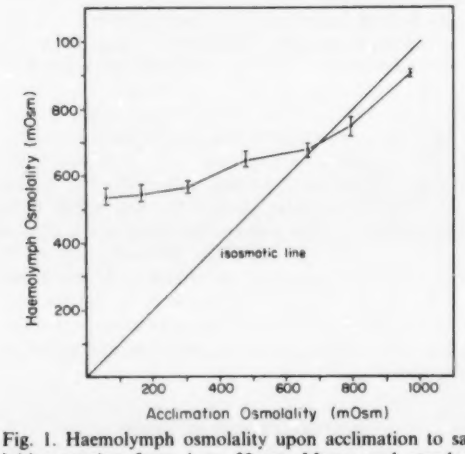

Fig. 1. Haemolymph osmolality upon acclimation to salinities ranging from 1 to 30 ppt. Means and standard deviations are plotted. The n for each mean is 5. The iso-osmotic line is plotted for reference.

To determine the rate of acclimation of haemolymph osmolality upon a sudden salinity change, crabs were acclimated for 3 days to 20 ppt and then placed in 1 ppt. These salinities were used because the crabs are approximately isosmotic at 20 ppt (Fig. 1) and a decrease to 0–1 ppt is a realistic salinity level after a heavy rain in estuarine areas inhabited by the crabs. The exact procedure was to acclimate 21 crabs to 20 ppt. Blood osmolality was initially determined for three crabs. The remaining crabs were then transferred into 1 ppt sea-water and three crabs were sampled thereafter at 1, 4, 8, 12, 24 and 48 hr.

The tests for the effects of Alachlor upon osmoregulation consisted of acclimating 18 crabs to 20 ppt for 3 days. Two separate groups of three crabs were initially sampled to indicate haemolymph osmolality upon acclimation to 20 ppt. Two groups of six crabs were then placed individually in separate finger bowls containing 50 ml of 25 ppm Alachlor made up in 1 ppt seawater. Haemolymph of three crabs was sampled at 12 and 24 hr. These times were selected because acclimation occurs within 8 hr and Alachlor effects on larvae are evident after 12 hr exposure time (Takacs *et al.*, 1988).

Respiration experiments

Since *R. harrisii* is a small crab (average adult weight about 0.4 g) and the water volume of the respirometer flasks was relatively large, it was necessary to measure oxygen uptake for groups of crabs. For each trial, seven crabs of approximately equal size were placed in a clean bottle filled with air-saturated sea-water (approximate volume = 350 ml). A fiberglass screen was inserted into the bottle for the animals to hold onto during the trial. The bottle was then sealed with a self-stirring oxygen probe. This apparatus was placed in a water bath maintaining a temperature of 24.5°C. Crabs were allowed to settle for 15 min, after which measurements of the oxygen concentration in the water were made every 15 min using an oxygen meter (Model 54, Yellow Springs Instruments Co., Inc.) attached to the bottle probe. The trial was terminated when the meter reading remained unchanged for 1 hr. The oxygen concentrations at each measurement interval throughout the test period were then converted to weight-specific oxygen consumption rate ($\dot{V}_{O_2}$) and the average oxygen tension for each 15 min interval.

To study the effects of salinity on respiration, four trials were initially run for separate groups of crabs acclimated for 1 week to 10 ppt. The study of the effect of a salinity decrease upon respiration used the same procedure, except that the oxygen consumption of the crabs was determined 24 hr after placement in 0 ppt water. This time was selected because crabs completed osmotic acclimation to a salinity decrease within 24 hr (Fig. 2).

Two experiments examined the effects of prior exposure to Alachlor and a sudden decrease in salinity upon respiration. First, animals were acclimated to 10 ppt sea-water for at least one week and then exposed to 10 or 25 ppm Alachlor for 24 hr. Crabs were then placed in herbicide-free 10 ppt sea-water for 0.5 hr, and respiration subsequently tested in 10 ppt sea-water. Testing in herbicide-free water reduced contamination of the respiratory equipment. Second, crabs were acclimated to 10 ppt for at least one week and then placed in 0 ppt water containing 25 ppm Alachlor for 24 hr. Crabs were then placed in herbicide-free 0 ppt water for 0.5 hr, after which respiration was measured in another volume of herbicide-free 0 ppt water.

Each experiment was replicated two or four times and all data were used to plot $\dot{V}_{O_2}$ at the various oxygen tensions because the crabs were all of approximately equal weight. A computer-derived 5th order polynomial best fit curve was drawn to approximate the respiratory rate as the oxygen tension decreased. Trials with different equations indicated

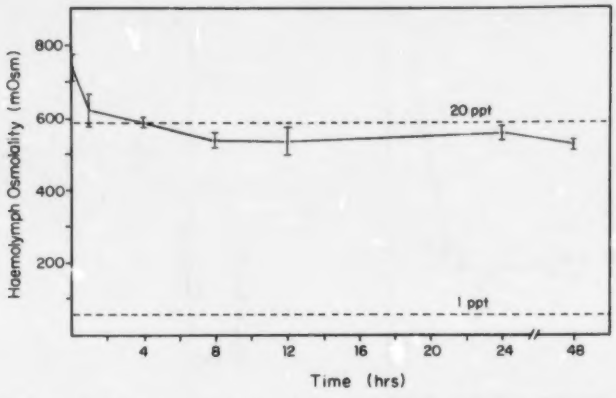

Fig. 2. The time course of adjustments in haemolymph osmolality upon a change in salinity from 20 to 1 ppt. Means and SDs are plotted. The n for each mean is 3. The horizontal dashed lines show the osmolality of the 20 and 1 ppt waters.

this polynomial provided the best fit to the data. For each set of trials, the average $\dot{V}_{O_2}$ for the independent respiration was calculated between oxygen tensions of 50 and 100 mm Hg. This interval eliminated the initial elevated $\dot{V}_{O_2}$ values caused by handling and disturbance of the animals when first placed in the bottle and also eliminated the possibility of including low $\dot{V}_{O_2}$ values during dependent respiration, because the critical oxygen tension (P_c) was below 50 mm Hg for these crabs. This average $\dot{V}_{O_2}$ line was also plotted on the graph. A vertical line was drawn from the point at which the best fit curve fell below the average $\dot{V}_{O_2}$ line to determine the P_c value.

RESULTS

Osmoregulation experiments

R. harrisii were acclimated to salinities ranging from 1 to 30 ppt. Haemolymph osmolality remained hyperosmotic until about 24 ppt (Fig. 1) and became hypoosmotic at higher salinities.

Crabs acclimated relatively rapidly upon a salinity decrease from 20 to 1 ppt (Fig. 2). Haemolymph osmolality decreased gradually for the first 8 hr and then stabilized around 550 mOsm. Mean haemolymph osmolalities at 8 hr and longer were not significantly different (Student's t-test) from the mean value upon acclimation for one week at 1 ppm (Fig. 1).

The test for the effect of Alachlor on osmoregulation indicated that neither 25 nor 50 ppm affected this process (Fig. 3). When the salinity decreased from 20 to 1 ppt in the presence of Alachlor, the haemolymph osmolality decreased and stabilised at about the same level as it did when Alachlor was not present (Fig. 2). Since *R. harrisii* is a strong hyperosmotic regulator (Fig. 2), a decline in the level of hyperosmotic regulation was predicted if osmoregulation was affected.

Respiration experiments

The respiratory pattern was the same under all test conditions (Figs 4–6). At high oxygen tensions there was a very high V_{O_2}, which probably resulted from an initial increase in activity upon being placed in the respirometer bottle. At progressively lower oxygen tension levels, the crabs displayed independent respiration followed by dependent respiration. At control conditions of 10 ppt and no Alachlor, the average $\dot{V}_{O_2}$ was 102.8 μl O_2/s/hr, and the crabs switched to dependent respiration at 15.9 mm Hg (Fig. 4; Table 1). Prior exposure of Alachlor in 10 ppt sea-water caused an increase in the $\dot{V}_{O_2}$ and P_c (Fig. 5; Table 1).

Upon a salinity decrease from 10 to 0 ppt, both the $\dot{V}_{O_2}$ and P_c were elevated (Fig. 6A) as compared to respiration in 10 ppt. However, when subjected to this salinity decrease in the presence of 25 ppm

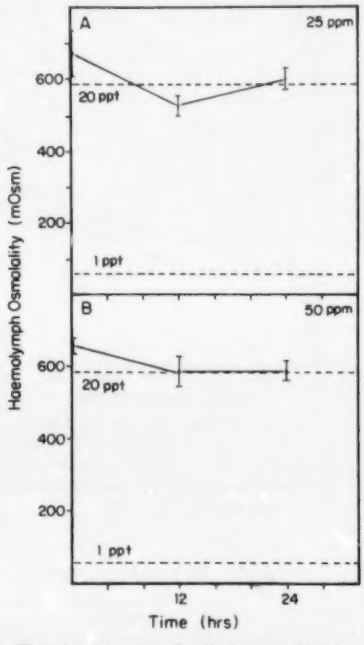

Fig. 3. The time course of adjustments in haemolymph osmolality upon a salinity change from 20 to 1 ppt in the presence of 25 ppm (A) and 50 ppm (B) Alachlor. Means and standard deviation are plotted. The n for each mean was 3. The horizontal dashed lines show the osmolality of the 20 ppt and 1 ppt waters.

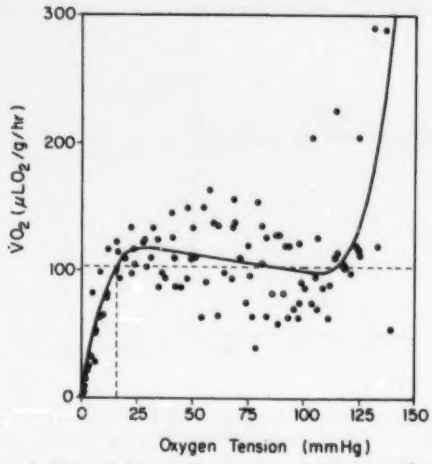

Fig. 4. The weight specific oxygen uptake rate ($\dot{V}_{O_2}$) at different oxygen tension in 10 ppt sea-water. Data from four trials are plotted. The average animal weight was 0.433 g. The horizontal dashed line represents the average $\dot{V}_{O_2}$ for oxygen tensions between 50 and 100 mm Hg. The vertical dashed line is the position of the critical oxygen tension (P_c).

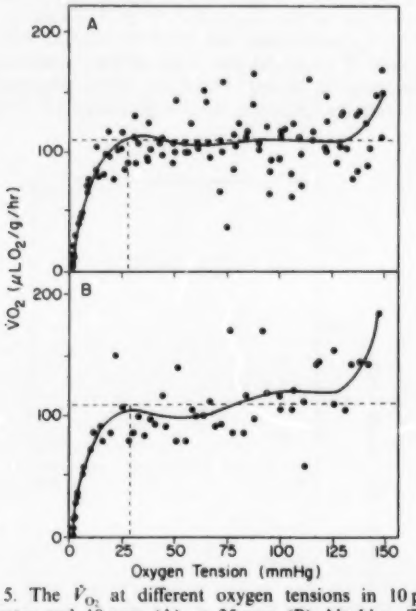

Fig. 5. The $\dot{V}_{O_2}$ at different oxygen tensions in 10 ppt sea-water and 10 ppm (A) or 25 ppm (B) Alachlor. The figure is plotted as in Fig. 4, except only two trials were plotted in (B). The average animal weight was 0.363 g in (A) and 0.34 g in (B).

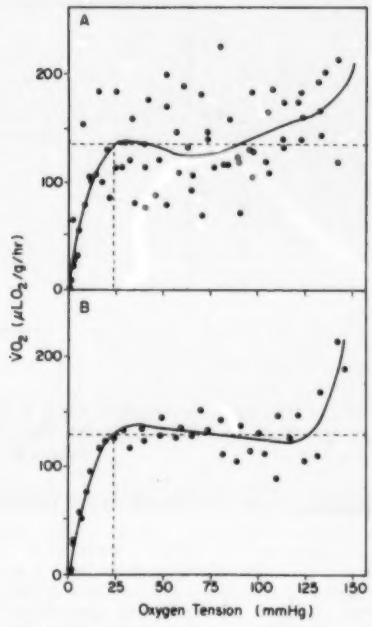

Fig. 6. The $\dot{V}_{O_2}$ at different oxygen tensions after a salinity change from 10 ppt to 0 ppt alone (A) for 24 hr. The same experiment was repeated in (B) except that crabs were also exposed to 25 ppm Alachlor. The figure is plotted as in Fig. 4, except only two trials were plotted in B. The average animal weight in (A) was 0.455 g and 0.435 in (B).

Alachlor, similar $\dot{V}_{O_2}$ and P_c values were observed (Fig. 6B; Table 1). Thus, Alachlor did not have any additional effect on the respiratory change upon a decrease in salinity.

DISCUSSION

A pattern of hyperosmotic regulation at low salinities and hypoosmotic regulation at high salinities is typical of estuarine grapsoid crabs (Barnes, 1967) and species living in high intertidal areas (Jones, 1941). However, most subtidal estuarine crustaceans are hyperosmotic at low salinity and become isosmotic at high salinities (e.g. *Callinectes sapidus*, Ballard and Abbott, 1969; *Panopeus herbstii*, Blasco and Forward, 1988).

In salinities ranging from 1 to about 24 ppt, *R. harrisii* is an osmoregulator, remaining hyperosmotic to the external environment. At salinities greater than about 24 ppt, the crabs displayed hypoosmotic regulation. These results agree with the previous study by

Table 1. The $\dot{V}_{O_2}$ and P_c for respiration under different salinities and Alachlor concentrations

Conditions		Number of trials	$\dot{V}_{O_2}$ (μl O_2/s/hr)	P_c (mm Hg)	Figure
Salinity	Alachlor				
10 ppt	0 ppm	4	102.8	15.9	4
10 ppt	10 ppm	4	109.1	28.0	5(A)
10 ppt	25 ppm	2	109.3	29.0	5(B)
0 ppt	0 ppm	4	134.1	24.1	6(A)
0 ppt	25 ppm	2	128.3	23.8	6(B)

Smith (1967). His measurements of haemolymph Cl^- concentration suggested that *R. harrisii* was a hyperosmotic regulator at salinities from 1 to 23 ppt and perhaps a hypoosmotic regulator at higher salinities.

R. harrisii acclimated its haemolymph osmolality to a decrease in salinity within 8 hr. This relatively rapid rate of adjustment is similar to that observed for a related species, *Panopeus herbstii* (Blasco and Forward, 1988), which stabilized its haemolymph osmolality within 4 hr upon an 8 ppt decrease in salinity. However, most crustaceans require 12–24 hr to stabilize their osmolality (*C. sapidus*, Tagatz, 1971; Engel and Nichols, 1977; *Callianassa jamaicense*, Felder, 1978). Neither 25 nor 50 ppm Alachlor affected the rate or level of adjustment by *R. harrisii* upon a dramatic salinity change from 20 to 1 ppt.

Upon exposure to declining oxygen tensions, *R. harrisii* shows the pattern of independent respiration at high tensions which changes to dependent respiration at low tensions. This pattern is typical of many crustaceans (Mangum and Van Winkle, 1973), especially those living in an environment having variable oxygen tensions.

Under possible normal conditions in an estuary (10 ppt, 0 ppm Alachlor), *Rhithropanopeus harrisii* has a P_c of 15.9 mm Hg (Table 1) and is in no danger of tissue damage caused by insufficient oxygen because normally the minimum measured oxygen tensions that occur in estuarine creeks in the summer at night are approximately 25 mm Hg (unpublished data, W. Kirby-Smith). The animals probably never switch to dependent respiration under normal salinities and thus their blood is always saturated with oxygen and no stress is caused to their tissues (Redmond, 1955). Under conditions of heavy rainfall in herbicide-free runoff creeks, however, the salinity may decrease to about 0 ppt, which causes the P_c to increase to approximately 24 mm Hg and the average $\dot{V}_{O_2}$ to increase by about 30%. This increase in respiration may represent the energy needed for increased hyperosmotic regulation. In this situation the respiratory volume and cardiac output may not saturate the blood to keep up with the elevated oxygen demands of the body, and the organism may experience anaerobic damage resulting in death (Arudpragasam and Naylor, 1964; Taylor and Butler, 1973; Taylor, 1976; Warner, 1977).

When exposed to both 10 and 25 ppm Alachlor in 10 ppt sea-water, both the $\dot{V}_{O_2}$ and P_c are elevated, which indicates the animals are experiencing sublethal stress. However, this situation is unrealistic because exposure to Alachlor will be accompanied by a salinity decrease due to rain water runoff. Under conditions of 25 ppm Alachlor and a salinity decrease, the average $\dot{V}_{O_2}$ and P_c increase by about 30% and 50%, respectively. This increase in P_c and $\dot{V}_{O_2}$ must be caused by the hypoosmotic stress because the $\dot{V}_{O_2}$ and P_c values are very similar to those upon a salinity decrease alone (Table 1). If the reduction in salinity occurs at night when there is no photosynthetic activity, the dissolved oxygen concentration may decline below the new critical oxygen tension and dependent respiration may result. If animals are prevented from reaching the air/water interface, or if they are unable to switch to anaerobic respiration for a long enough duration, then the animals may be stressed past their limits. This situation may explain the observed deaths of *R. harrisii*, which prompted this study.

Runoff events expose estuarine animals to low salinity water, which can potentially contain high concentrations of herbicides. Respiration and osmoregulation of adult *R. harrisii* are affected by rapid decreases in salinity but Alachlor did not affect hyperosmotic regulation or elevate respiration beyond the level due to the salinity decrease alone. Test concentrations of Alachlor were at or above the LC_{50} levels for larvae (Takacs *et al.*, 1988) and the same order of magnitude of the highest levels found in drainage areas (Skaggs *et al.*, 1980). Thus, it is unlikely that Alachlor caused the observed death of *R. harrisii* in the field after a runoff event which prompted this study. A more likely explanation is that death resulted from elevated respiration in response to the salinity reduction and reduced oxygen levels at night (Kenney *et al.*, 1988).

Acknowledgements—This study was funded in part by the Environmental Protection Agency, Contract No. CR 813415-01. We thank Monsanto Agricultural Products, Inc. for providing the technical grade Alachlor.

REFERENCES

Arudpragasam K. D. and Naylor E. (1964) Gill ventilation volumes, oxygen consumption and respiratory rhythms in *Carcinus maenas* (L). *J. exp. Biol.* **41**, 309–321.

Ballard B. S. and Abbott W. (1969) Osmotic accommodation in *Callinectes sapidus* (Rathbun). *Comp. Biochem. Physiol.* **29**, 671–687.

Barnes R. S. K. (1967) The osmotic behaviour of a number of grapsoid crabs with respect to their differential penetration of an estuarine system. *J. exp. Biol.* **47**, 535–551.

Blasco E. and Forward R. (1988) Osmoregulation of the xanthid crab *Panopeus herbstii*. *Comp. Biochem. Physiol.* **90A**, 135–139.

Cameron J. N. and Mangum C. P. (1983) Environmental adaptation of the respiratory system: Ventilation, circulation and oxygen transport. In *The Biology of Crustacea: Environmental Adaptation* (Edited by Vernberg F. J. and Vernberg W. B.), Vol. 8, pp. 43–62. Academic Press, New York.

Engel D. W. and Nichols C. D. (1977) A method for continuous *in vivo* measurements of haemolymph conductivity in crabs. *J. exp. Mar. Biol. Ecol.* **26**, 203–209.

Felder D. L. (1978) Osmotic and ionic regulation in several western callianassidae (Crustacea, decapoda, Thalassinidae). *Biol. Bull. (Woods Hole, Mass.)* **154**, 409–429.

Gilles R. and Pequeux A. (1983) Interaction of chemical and osmotic regulation with the environment. In *The Biology of Crustacea: Environmental Adaptation* (Edited by Vernberg F. J. and Vernberg W. B.), Vol. 8, pp. 109–178. Academic Press, New York.

Hill R. W. (1976) *Comparative Physiology of Animals: An Environmental Approach*, pp. 1–656. Harper and Row, New York.

Jones L. L. (1941) Osmotic regulation of several crabs of the Pacific coast of North America. *J. Cell comp. Physiol.* **18**, 79–92.

Kenney B. E., Litaker W., Duke C. S. and Ramus J. (1988) Community oxygen metabolism in a shallow tidal estuary. *Estuarine, Coastal and Shelf Science* **27**, 33–43.

Mangum C. and Van Winkle W. (1973) Responses of

aquatic invertebrates to declining oxygen conditions. *Am. Zool.* **13**, 529–541.

Monsanto Company (1984) *Lasso: Herbicide by Monsanto–Complete Directions for Use*. St. Louis, MO.

Redmond J. R. (1955) The respiratory function of hemocyanin in Crustacea. *J. Cellular comp. Physiol.* **46**, 209–247.

Skaggs R. W., Gilliam J. W., Sheets T. J. and Barnes J. S. (1980) Effects of agricultural land development on drainage waters in the North Carolina tide water region. Water Resources Institute of the University of North Carolina. Report No. 159 (August 1980).

Smith R. I. (1967) Osmotic regulation and adaptive reduction of water permeability in a brackish-water crab, *Rhithropanopeus harrisii* (Brachyura, Xanthidae). *Biol. Bull. (Woods Hole, Mass.)* **133**, 643–658.

Takacs R., Forward R. B. and Kirby-Smith W. (1988) Effects of the herbicide Alachlor on larvel development of the mud-crab, *Rhithropanopeus harrisii* (Gould). *Estuaries* **11**, 79–82.

Tagatz M. (1971) Osmoregulatory ability of the blue crab in different temperature–salinity combinations. *Chesapeake Sci.* **12**, 14–17.

Taylor A. C. (1976) The respiratory responses of *Carcinus maenas* to declining oxygen tension. *J. exp. Biol.* **65**, 309–322.

Taylor E. W. and Butler P. J. (1973) The behavior and physiological responses of the shore crab *Carcinus maenas* during changes in environmental oxygen tension. *Neth. J. Sea Res.* **7**, 496–505.

Vernberg F. J. (1983) Respiratory adaptations. In *The Biology of Crustacea: Environmental Adaptation* (Edited by Vernberg F. J. and Vernberg W. B.), Vol. 8, pp. 1–42. Academic Press, New York.

Warner G. F. (1977) *The Biology of Crabs*. Elek Science, London.

Wauchope R. D. (1978) The pesticide content of surface water draining from agricultural fields—A review. *Journal of Environmental Quality* **7**, 458–472.

Weed Science Society of America (1979) *Herbicide Handbook*, 4th edn, pp. 1–479. Weed Science Society of America, Champaign, Ilinois.

Williams A. B. (1984) *Shrimps, Lobsters and Crabs of the Atlantic Coast of Eastern United States, Maine to Florida*, pp. 1–550. Smithsonian Institution Press, Washington DC.

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.